ADDITION OF PERFUME FIXATIVES TO MOSQUITO REPELLENTS TO INCREASE PROTECTION TIME

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ABSTRACT. Perfume fixatives were examined as extenders of protection time of insect repellents. We investigated 7 synthetic musks combined in 3 different ratios with 4 insect repellents. Four of 7 fixatives investigated gave increased protection with deet, depending on formulation ratios. None of these fixatives proved effective with dimethyl phthalate, ethyl hexanediol or Indalone®. Their probable mode of action is discussed.

INTRODUCTION. N,N-diethyl-m-toluamide (deet) is the mosquito repellent of choice for military and civilian use in the U.S.A. Ethyl hexanediol and dimethyl phthalate formulations are also marketed. However, these repellents do not protect against mosquitoes for more than a few hours. Smith et al. (1963) reported 5.6–6.2 hr protection against *Aedes aegypti* with 10% deet on the forearms of 3 subjects. Altman (1969) obtained only 2.0–2.2 hr protection against *Anopheles albimanus* with the same amount of deet. Gilbert et al. (1970) tested 10% deet against *Culex pipiens quinquefasciatus* and obtained protection for 2.2 hr in 10 tests. These studies show that the protection obtained with deet against other mosquito species may be considerably shorter than against *A. aegypti*. In such cases, the repellent must be applied more frequently. The required frequency of application also depends on ambient temperature and air velocity. Khan et al. (1973) demonstrated a highly significant ($P<0.01$) negative correlation ($r=-0.96$) between repellent protection times and temperature; also protection time decreased to ⅓ in the presence of airflow (3.2m/sec) compared to protection time without airflow. Considering these limiting factors, prolonging effectiveness of existing mosquito repellents is needed until the search for new longer-lasting mosquito repellents is successful.

Fixatives are commonly used in perfume formulations. Since most fragrances in a scent are volatile, the perfume would normally be evanescent. Perfumes are required to be reasonably long-lasting and this objective is gained by the addition of a fixative, a relatively non-volatile constituent which retains or fixes the odor of the other components. Such fixatives include musk, civet, and ambergris. In the following we describe potentiation of existing repellents with certain perfume fixatives added.

Methods and Materials. We combined 7 aromatic nitrogen compounds with deet, ethyl hexanediol, dimethyl phthalate, and butyl-3,4-dihydro-2, 2-dimethyl-4-oxo-2*H* pyran-6-carboxylate (Indalone®) to examine their effects on protection time. Repellents were applied in 2 different dosages, each dose combined with each fixative at 3 different ratios. The formulation was applied to a 5 x 25 cm area on the ventral surface of one forearm of male volunteers and a similar quantity of the repellent without fixative on the contralateral forearm. The test method was modified from that of Granett (1940). A long plastic sleeve with a cut out area matching the marked skin test site was pulled over the forearm and held in place by means of a wire clamp, thus exposing only the treated surface. The hand end of the sleeve was sealed to serve as a glove. The treated skin area was exposed to approximately 500 female *A. aegypti* in a 1 cu ft cage through a long sleeve to introduce the forearm. The mosquitoes in this experiment had been fed 5% sugar solution only and had not had any previous blood meal. The arm was kept in the cage for 3 min and then withdrawn. Exposures were repeated every 30 min until a total of 2 bites were obtained. The time from application to 2 bites was designated as the protection time. The 7 synthetic musks studied were: Tibetene® (2, 6-dinitro-3,4,5-triethyl-t-butyl benzene), Moskene® (4,6-dinitro-1,1,3,3,5-pentamethyl indan), musk ambrette (2,4-dinitro-3-methyl-6-t-butyl anisole), musk xylol (5-t-butyl-2,4,6-trinitro-m-xylene), musk ketone (4-t-butyl-3,5-dinitro-2,6-dimethyl acetophenone), benzylcinnamate, and givambrol (2,4-di-t-butyl-5-methoxybenzaldehyde). All tests were performed in a room kept at $27 \pm 1°$ C and 60% RH.

Results and Discussion. Table 1 gives the results of those fixatives found effective in enhancing the protection time of deet. They were the musks, Tibetene®, ambrette, givambrol, and xylol. The musks Moskene®, ketone, and benzylcinnamate were ineffective. With deet applied at 0.16 mg/cm^2, mixed with Tibetene®, protection time was enhanced significantly with the 3 ratios examined and was directly proportional to the quantity of musk in the formulation. Tibetene® also enhanced the efficacy of deet significantly in all 3 ratios when the dosage of the latter was increased to 0.32 mg/cm^2. Musk ambrette was effective only in 1 ratio (1:1) when deet was used at 0.16 mg/cm^2. With a higher concentration of deet (0.32 mg/cm^2), it was effective at 1:1 and 1:1.5 ratios. Givambrol was not effective with deet applied at 0.16 mg/cm^2, but with deet applied at 0.32 mg/cm^2 it gave a significant increase in protection time at the 1:0.5 and 1.1 ratios. Musk xylol was effective only when deet was used at 0.32 mg/cm^2 mixed with the musk at half its strength. Formulation of musks with dimethyl phthalate, ethyl hexanediol and Indalone® did not enhance their protection times in 4 trials done with each repellent.

In the past, several workers have attempted to increase repellent protection time by formulation with various other materials. Formulations containing zinc oxide were effective longer than pure repellent at the same dilution (Smith, 1970). Smith, Kline and French Laboratories prepared more than 2,000 formulations, 252 of which were tested against mosquitoes by the USDA Laboratory at Orlando, Florida. The formulations did not prove to be better than full strength repellents and there was no improvement in cosmetic acceptability (Smith, 1970). Kharitonova and Koshkina (1969) combined 14 polymers and fixatives of perfumes with dimethyl phthalate and reported prolongation of its protection time with *A. aegypti* under laboratory conditions. In a subsequent study, Koshkina and Kharitonova (1970) used ethyl cellulose as a repellent prolongator, without success. Our own studies with ethyl cellulose confirm their results. The increase in repellent protection time with synthetic musks, as reported here is encouraging and suggests exploring this potentially promising field further. Some of the formulations were tested independently at the Letterman Army Institute of Research, San Francisco, hence the greater number of replicates for these than others (Table 1).

Table 1. Mean protection time against mosquito bites obtained with deet and its formulation with musks mixed in different ratios.

Deet (control) (mg/cm^2)	No. of trials	Musk	Formulation (mg/cm^2) repellent/musk	Protection time (hr) Deet (control)	Deet+ musk	% Increase	Significance (P)
0.16	16	Tibetene®	0.16+0.16	3.1	4.0	29	<0.05
0.16	9	"	0.16+0.32	1.7	2.5	47	<0.05
0.16	9	"	0.16+0.48	1.6	3.0	88	<0.05
0.32	17	"	0.32+0.16	4.5	5.6	24	<0.05
0.32	20	"	0.32+0.32	5.8	6.5	12	<0.01
0.32	27	"	0.32+0.48	5.7	6.7	18	<0.01
0.16	15	Ambrette	0.16+0.16	2.7	3.6	33	<0.01
0.32	4	"	0.32+0.32	6.5	8.1	25	<0.01
0.32	5	"	0.32+0.48	8.9	10.3	16	<0.05
0.32	14	Givambrol	0.32+0.16	5.1	7.0	37	<0.01
0.32	8	"	0.32+0.32	6.0	8.6	43	<0.05
0.32	4	Musk xylol	0.32+0.16	6.4	8.3	30	<0.05

The mode of action of fixatives is inadequately understood. It has been suggested that fixatives reduce the vapor pressure of odorous material. Moncrieff (1967) thought that molecules of odorous material are adsorbed on molecules of fixative, or are loosely combined by means of residual valences with fixatives. The fixatives perhaps play the role of an anchor to escaping repellent molecules. The effectiveness of only particular fixatives and not all in enhancing protection time is intriguing and defies simple explanation. Also, enhancement of the protection period in some subjects and ineffectiveness in others suggests a complicating factor of skin interaction with the formulations. It appears that on some skin surfaces it binds better than on others. In *in vitro* studies, Kurtz (1972) found musk Tibetene® considerably reduced evaporation of ^{14}C-labeled deet from a glass surface. Further, no increase in protection with repellents other than deet suggests a chemical affinity of musks for deet. Their role in anchoring deet molecules to the skin, or reducing the evaporation or penetration of deet in the skin, requires investigation. Work with other fixatives is in progress.

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